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ELECTROPHORETIC PATTERNS OF PROTEINS IN SPANISH MACKEREL (SCOMBEROMORUS MACULATUS)

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INTRODUCTION

Spanish mackerel, Scomberomorus maculatus, is an important recreational and commercial fishery resource along the Atlantic and Gulf of Mexico coasts of the United States. The species occurs from the Gulf of Maine through the Florida Keys and in the Gulf of Mexico (Berrien and Finan 1977, Collette and Russo 1979). This fish is a coastal pelagic species which makes seasonal migrations along the Atlantic seaboard and in the Gulf of Mexico (Anonymous 1980). Three major migration routes paralleling the coast lines of the United States and Mexico have been hypothesized: (1) along the Mexican-Texas coast; (2) along the northern Gulf of Mexico coast and west coast of Florida; and (3) along the Atlantic coast (Berrien and Finan 1977, Beaumariage 1970, Wollam 1970, Sutherland and Fable 1980). The species is believed to have winter grounds off south Florida and in the Campeche-Yucatan area (Anonymous 1980, Mendizabal and Vasconcelos cited by Sutherland and Fable 1980).

The number of discrete breeding populations of Spanish mackerel stocks is unknown. The determination of the number and range of stocks is important for proper management of the resources. One of the most promising methods to separate and delineate stocks is the analysis of genetic variants of proteins. This method has been useful in separating both marine and fresh water fish populations, such as Pacific hake, Merluccius productus, (Utter and Hodgins 1969), Atlantic cod, Gadus morhua, (Jamieson and Turner 1978) and brown trout, Salmo trutta, (Allendorf et al. 1976). Biochemical differences have been reported for Spanish mackerel from the Atlantic and Gulf of Mexico coasts (Collette and Chittenden cited by Anonymous 1980).

The purpose of this paper is to report the biochemical variations and electrophoretic patterns found in tissues of Spanish mackerel using starch gel electrophoresis. This examination was performed in order to (1) find biochemical variants which may be useful to identify stocks and (2) estimate the amount of genetic variation in this species.

METHODS AND MATERIALS

One hundred mature Spanish mackerel (270 mm to 510 mm fork length) were collected from northwest Florida by commercial seine and hook and line from August to October 1980. These fish were frozen until tested. The following tissues were removed from the frozen fish: skeletal (white) muscle, liver, heart, and vitreous fluid. Tissue extracts (except vitreous fluid) were prepared by mixing equal volumes of tissue and distilled water into uniform pastes with glass rods. The vitreous fluid was tested without dilution. The extracts were centrifuged for five minutes, after which the supernatant was drawn onto 4 mm x 8 mm filter paper inserts (Schleicher and Schuell grade S&S No. 470[1/]). Electrophoretic separation of the extracts was performed following the methods of Kristjansson (1963) with modifications found in Johnson et al. (1972). The electrophoretic buffers (Table 1) were those of:

1/ Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

(A) Markert and Faulhaber (1965); (B) Ridgway et al. (1970); and (C) N-(3-aminopropyl)-morpholine-citrate (pH6.1) buffer of Clayton and Tretiak (1972). The gel consisted of 35 g starch (Electrostarch lot 303) plus 250 ml of buffer. The amperage during electrophoresis was kept below 50 ma and the voltage varied between 100 and 400 v depending on the buffers. After electrophoresis, the gels were sliced into four horizontal sections and stained for the various proteins using methods presented in Table 2.

RESULTS AND DISCUSSIONS

The results of this investigation are presented in two parts: electrophoretic patterns and polymorphisms. The resolution of the electrophoretic patterns was best from skeletal muscle.

Part 1. Electrophoretic patterns:

The electrophoretic patterns found for the various proteins are summarized in Table 3. Also included are my interpretations of the number of loci expressed, the subunit structure, and the best buffer system and tissue for electrophoretic resolution. These interpretations are based on the generally accepted explanations of electrophoretic patterns (see Shaw 1964, Hubby and Lewontin 1966, Lewontin and Hubby 1966, Brewer 1970, Ayala et al. 1970, Powell 1975, Harris and Hopkinson 1976). The proposed number of loci based on the number of bands observed in the electropherograms is conservative; that is, if protein bands are found with identical mobility in different tissues, they are considered to be the product of the same locus and are counted only once.

Part 2. Polymorphisms:

Electrophoretic variants whose patterns appeared to reflect genetic polymorphisms were found in ten enzyme systems. These polymorphic systems are summarized in Table 4 along with Chi square (X^2) analyses to determine if the systems agree with Hardy-Weinberg expectations. The values are presented to give the readers an approximate indication of the degree of variation found for a particular enzyme system and are not meant to represent the actual values for Spanish mackerel from northwest Florida. The allelic identifiers such as F, S, and VS and A, B, and C are arbitrary designations representing the alleles' positions on the electropherogram. F is more anodal than VS and the same applies to the A, B, C system. The allelic identifiers are also presented in numeric form which are designed according to the relative electrophoretic mobility of the proteins. The most common allele is designated as 100. This value represents the distance from the origin that the protein has migrated. The other alleles of the system are assigned numeric values representing their mobility in relation to the most common allele (i.e., 50 would represent an allele that migrated only half the distance from the origin as the most common allele). Each of the polymorphic systems is discussed in more detail below.

Alpha-glycerophosphate dehydrogenase (GPDH)

A single polymorphic zone of activity was found for GPDH. This system had two alleles (F = 100 and S = 64). The heterozygote possessed a three banded pattern which is typical of a dimeric enzyme. The phenotypic distribution was in agreement with Hardy-Weinberg expectations. (X^2 = 1.64; d.f. = 1; .2>P>.1).

Lactate dehydrogenase (LDH)

Three LDH loci (A, B, and C) were expressed in the Spanish mackerel tissues (system of nomenclature of Shaklee et al. 1973). LDH_B was polymorphic in Spanish mackerel. All tissues expressed the LDH_B polymorphism. This system had two alleles (F = 200 and S = 100) and the single observed heterozygote showed a five banded tetrameric pattern.

The LDH_C locus was detected only in the vitreous fluid. The locus was expressed as a single anodal band which migrated more to the anode than the other two loci (A and B).

Malate dehydrogenase (MDH)

Two zones of activity were found for MDH. The most anodal zone (Zone I) was polymorphic with two alleles (F = 100 and S = 85), and the heterozygote was represented by a three banded pattern which is indicative of a dimeric enzyme. Only the homozygote FF (100/100) and the heterozygote FS (100/85) were found in the collection. The homozygote SS (85/85) was not expected because of its low expected frequency of occurrence (0.015).

Malic enzyme (ME)

Two polymorphic zones (I and II) of activity were found in Spanish mackerel. The most anodal zone (I) had a two allele (F = 110 and S = 100) system, and the heterozygote pattern was the two banded pattern of a monomeric enzyme. This enzyme is listed by Harris and Hopkinson (1976) as having a tetrameric subunit structure in man; thus, the subunit structure of this enzyme in Spanish mackerel may not be that interpreted from its pattern in the electropherogram.

The less anodal ME zone of activity (II) was also polymorphic, and two phenotypes were found (FF = 100/100 and SS = 75/75). The third phenotype (FS = 100/75) was expected to be rare and was not found.

Glutamic dehydrogenase (GDH)

GDH was expressed as a single polymorphic zone of activity. This variant system had two alleles (F = 100 and S = 75) and the typical monomeric heterozygote pattern. The phenotypic distribution was not in agreement with Hardy-Weinberg expectation ($X^2 = 31.09$; d.f. = 1; $P<0.001$).

Esterase (ES)

The substrate used for examination of esterase in Spanish mackerel was alpha napthyl acetate which resulted in the expression of four zones of activity (I-IV). The least anodal zone (IV) was found to be polymorphic with four alleles (A' = 106, A = 105, B = 100, and C = 95). The two banded heterozygous pattern was that of a monomeric system. The esterase phenotypic distribution was not in agreement with the Hardy-Weinberg expectation ($X^2 = 25.06$; d.f. = 1; $P<.001$).

An additional variant esterase system was found in liver extracts. However, the electrophoretic resolution of this system was not suitable for evaluation.

Peptidase (PEP)

Two anodal zones (I and II) of activity were found for peptidase using L-glycyl-L-leucine as the substrate. The most anodal zone (I) was polymorphic with a two allele (F = 100 and S = 90) monomeric system. The phenotypic distribution did not agree with the Hardy-Weinberg distribution ($X^2 = 16.71$; d.f. = 1; $P<.001$).

Adenoside deaminase (ADA)

Adenoside deaminase was expressed as two anodal zones of activity (I and II). The least anodal zone (II) was polymorphic showing the patterns of a three allele (F = 111, S = 100, and VS = 90) monomeric system. All phenotypes were observed except the heterozygote FVS (111/90). This pattern (FVS = 111/90) was not expected to be observed in a sample size of 100 fish because of its low expected frequency of occurrence (less than 0.004). The phenotypic distribution did not agree with the expected Hardy-Weinberg distribution (X^2 = 37.1; d.f. = 1; P<.001).

Phosphoglucose isomerase (PGI)

Seven zones of activity were found for PGI on the electropherograms of muscle extracts. Only the most anodal zone (I) was polymorphic. This polymorphic system consisted of a two allele (F = 110 and S = 100) dimeric system. The phenotypic distribution agreed with the expected distribution derived from Hardy-Weinberg expectations (X^2 = 0.00; d.f. = 1; P>.99).

CONCLUSION

In this study 44 loci were examined in Spanish mackerel, of which 22.7% were polymorphic. This relatively high amount of biochemical variation indicates that when additional protein systems are examined more variation can be expected to be found.

Four of the ten polymorphic systems that were found in the Spanish mackerel collection deviated from Hardy-Weinberg expectations. This amount of deviation may be the result of mixing of stocks, natural selection, or a combination of these two causes. Additional studies of the biochemical variant systems of Spanish mackerel are in progress.

The proportion of genome heterozygous per individual in the present collection was estimated to be 0.06 and the maximum proportion of the genome heterozygous to be 0.11 (calculated according to the procedure of Lewontin and Hubby (1966)). These values are in the middle of the range of values reported for other fish (Fuerst et al. 1977), which indicates that one can expect Spanish mackerel to have as much variation as other teleosts.

The biochemical variant systems identified in this study and the high potential for finding additional systems in Spanish mackerel suggested that separation of stock by this technique is very feasible. If one or more systems are identified as having significantly different allele frequencies (agreeing with Hardy-Weinberg expectations) in separate locations of the Spanish mackerel range, then we have a good basis for expecting that the species consists of more than one stock.

SUMMARY

An electrophoretic examination of tissues (skeletal muscle, heart, liver, and vitreous fluid) from 100 Spanish mackerel from northwest Florida was performed using starch gel electrophoretic techniques. Ten of the 44 loci studied were found to be polymorphic. The variant systems were alpha-glycerophosphate dehydrogenase, lactate dehydrogenase (B locus), malate dehydrogenase, malic enzyme (two variant systems), glutamic dehydrogenase, esterase, peptidase, adenoside deaminase, and phosphoglucose isomerase.

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Table 1. Electrophoretic buffers used in this study.

(A) Buffer by Markert and Faulhaber (1965) (pH 8.7)

Stock Solution

Tris (0.9M)
Boric acid (0.5M)
Na EDTA (disodium ethylenediamine tetraacetate) (0.02M)

A 1:20 dilution of this stock solution was used for gel preparation and a 1:5 dilution was used for the electrode buffer. During electrophoresis 250-300v were applied to the gel.

(B) Buffer by Ridgway et al. (1970)

Gel Buffer (pH 8.5)

Tris [Tris (hydroxymethyl) aminoethane] (0.03M)
Citric acid (0.005M)

Electrode Buffer (pH 8.1)

Lithium hydroxide (0.06M)
Boric acid (0.3M)

Gels were made using 90% gel buffer and 1% electrode buffer. During electrophoresis 200-250V were applied to the gel.

(C) Buffer by Clayton and Tretiak (1972)

Stock Solution

Citric acid (0.4M)
Adjusted to pH 6.1 with N-(3-Aminopropyl)-morpholine

A 1:20 dilution of this stock solution was used for gel preparation and used undiluted for the electrode buffer. During electrophoresis 200-250v were applied to the gel.

Table 2. Composition of staining solutions used for electrophoretic analysis of proteins. These solutions were adopted from Shaw and Prasad (1970) unless noted otherwise. With the exceptions of AAT, ADA, and NP all of the stains listed below were mixed in 100 ml of a tris-citrate buffer (0.03 M tris, 0.005 M citric acid, pH 8.5).

PROTEIN	ENZYME COMMISSION NUMBER	ABBREVIATION	NBT[1/] and PMS	Cofactor[2/] (5mg)	OTHER COMPONENTS
Alcohol dehydrogenase	1.1.1.1	ADH	+	NAD	5 ml 95% ethanol
Alpha-glycerophosphate dehydrogenase	1.1.1.8	GPDH	+	NAD	50 mg DL-alpha-glycerophosphate
Sorbitol dehydrogenase	1.1.1.14	SORDH	+	NAD	100 mg D-sorbitol
Lactate dehydrogenase	1.1.1.27	LDH	+	NAD	1 ml DL-Lactic acid (60% Syrup)
Malate dehydrogenase	1.1.1.37	MDH	+	NAD	100 mg DL-malic acid
Malic enzyme	1.1.1.40	ME	+	NADP	100 mg DL-malic acid
6-phosphogluconate dehydrogenase	1.1.1.44	PGDH	+	NADP	20 mg Na-6-phosphogluconate
Xanthine dehydrogenase (from Brewer 1970)	1.2.3.2	XDH	+	NAD	5 mg - Hypoxanthine
Glutamic dehydrogenase (from Brewer 1970)	1.4.1.2	GDH	+	NAD	30 mg L-glutamic acid
Superoxide dismutase	1.15.1.1	SOD	+	-	-
Aspartate aminotransferase (from Johnson et al. 1972).	2.6.1.1	AAT	-	-	100 ml H_2O 0.8 g alpha-ketoglutaric acid 2.7 g aspartic acid 14 g Na_2 HPO_4 10 g 45% polyvinylpyrrolidinone 10 g Fast Garnet GBC salt 1 g Na EDTA

Table 2. (con't)

PROTEIN	ENZYME COMMISSION NUMBER	ABBREV-IATION	NBT[1/] and PMS	Cofactor[2/] (5mg)	OTHER COMPONENTS
Phosphoglucomutase	2.7.5.1	PGM	+	NADP	20 mg K-glucose-1-phosphate 20 units glucose-6-phosphate dehydrogenase 1 mg (or less) glucose-1,6-diphosphate
Esterase	3.1.1.1	ES	-	-	5 ml 1% alpha napthyl acetate in acetone 10 mg Fast Blue BB salt
Peptidase	3.4.3.1	PEP	-	-	10 mg O-dianisidine in acetone 5 mg L-amino acid oxidase 10 mg peroxidase 20 mg glycyl L-leucine
Adenoside deaminase (from Harris and Hopkinson 1976)	3.5.4.4	ADA	+	-	25 ml 0.05 M phosphate buffer pH 7.5 15 mg adenosine 25 units Nucleoside phosphorylase 4 units xanthine oxidase
Fumerase	4.2.1.2	FUM	+	NAD	16 mg Na funarate 60 units malate dehydrogenase
Phosphomanoisomerase (from Schaal and Anderson 1974)	5.3.1.8	PMI	+	NADP	20 mg Na-manose-6-phosphate 10 units glucose-6-phosphate dehydrogenase 20 units glucose phosphate isomerase
Phosphoglucose isomerase	5.3.1.9	PGI	+	NADP	50 mg Na-fructose-6-phosphate 20 units glucose-6-phosphate dehydrogenase
Succinate dehydrogenase (from Brewer 1970)	-	SDH	+	NAD	100 mg Na-succinate 25 mg Adenosine triphosphate
Nonspecific protein	-	NP	-	-	0.1% nigrosin-buffalo black in 1:4:5 solution of acetic acid:methanol:water Destain with same 1:4:5 solution

1/ A (+) in this column indicates the presence of both 5 mg P-nitro blue tetrazolium (NBT) and 10 mg phenazine methosulfate (PMS).

2/ Cofactors NAD is B-diphosphopyridine nucleotide
NADP is triphosphopyridine nucleotide with 1 mg M_gCl_2

Table 3. Results of electrophoretic examination of tissues of Spanish mackerel (*Scomberomorus maculatus*).

Protein 1/	Number of bands in starch gel	Proposed number of loci	Proposed number of alleles per loci	Number of polymorphic loci	Subunit 2/ structure	Tissue 3/	Buffer system 4/
ADH	1	1	1	0	-	M	C
GPDH	1 or 3	1	2	1	dimeric	M	A
SORDH	1	1	1	0	-	H	C
LDH_A	1	1	1	0	-	M	B,C
LDH_B	1 or 5	1	1	1	tetrameric	M	B,C
LDH_C	1	1	1	0	-	V	B,C
$MDH_{zone\ I}$	1 or 3	1	2	1	dimeric	M	C
$MDH_{zone\ II}$	1	1	1	0	-	M	C
$ME_{zone\ I}$	1 or 3	1	2	1	dimeric	M	C
$ME_{zone\ II}$	1 or 2	1	2	1	monomeric	M	C
PGDH	1	1	1	0	-	M	A
XDH	1	1	1	0	-	M	A
GDH	1 or 2	1	2	1	monomeric	M,H	C
SOD	1	1	1	0	-	M	B
AAT	1	1	1	0	-	M	A
PGM	1	1	1	0	-	M	B
$ES_{zones\ I-III}$	3	3	1	0	-	M	B
$ES_{zone\ IV}$	1 or 2	1	4	1	monomeric	M	B
$PEP_{zone\ I}$	1 or 2	1	2	1	monomeric	M	A,B,C
$PEP_{zone\ II}$	1	1	1	0	-	M	A,B,C
$ADA_{zone\ I}$	1	1	1	0	-	M	A
$ADA_{zone\ II}$	1 or 2	1	3	1	monomeric	M	A
FUM	1	5	1	0	-	M	C
PMI	5	1	1	0	-	M	B
$PGI_{zone\ I}$	1 or 3	1	2	1	dimeric	M	A
$PGI_{zones\ II-VII}$	6	6	1	0	-	M	A
SDH	1	1	1	0	-	M	C
NP	6	6	1	0	-	M	B

1/ See Table 2 for definition of abbreviations. Identifiers such as zone I refers to position of bands on starch gel. Zone I is the most anodal and zone VII the least anodal.

2/ - means that no determination of the subunit structure can be made based on observed electrophoretic patterns.

3/ Tissue of best resolution: M = skeletal muscle; V = vitreous fluid; and H = heart.

4/ See Table 1 for definition of abbreviations.

Table 4. Summary of polymorphic systems (and analysis) found in Spanish mackerel from northwest Florida.

Polymorphic[1/] system	Number of Phenotypes			Allele Frequency		x^2	P
GPDH	FF=41	FS=42	SS=17	F=.62	S=.38	1.64	.2>P>.1
LDH_B	FF=0	FS=1	SS=99	F=.01	S=.99	-	-
$MDH_{zone\ I}$	FF=97	FS=3	SS=0	F=.99	S=.01	-	-
$ME_{zone\ I}$	FF=2	FS=13	SS=85	F=.08	S=.92	1.07	.5>P>.3
$ME_{zone\ II}$	FF=99	FS=0	SS=1	F=.99	S=.01	-	-
GDH	FF=71	FS=16	SS=13	F=.79	S=.21	31.09	P<.001
$ES_{zone\ IV}$[2/]	AA=14	AB=16	BB=70	A=.22	B=.78	25.06	P<.001
$PEP_{zone\ I}$[3/]	FF=6	FS=2	SS=92	F=.93	S=.07	16.71	P<.001
$ADA_{zone\ II}$	FF=4	FS=80	SS=16	F=.44	S=.56	37.10	P<.001
$PGI_{zone\ I}$	FF=24	FS=50	SS=26	F=.49	S=.51	0.00	P>.99

1/ See Table 2 for definitions of abbreviations.

2/ Because of the rareness of A' and C alleles, the A' and A alleles are combined as A and the B and C alleles are combined as B for analysis

3/ Because of the rareness of VS allele, the VS and S alleles are combined as S for analysis.